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# SL65.0155, a 5-HT4 PARTIAL AGONIST REVERSES MEMORY DEFICITS INDUCED BY β25-35 AMYLOID PEPTIDE I.C.V. ADMINISTRATION IN MICE

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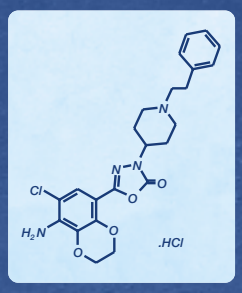
## Introduction

- Alzheimer's disease (AD) is a neurodegenerative disorder characterized notably by cognitive decline.
- 5-HT4 receptor agonists have been shown recently to improve learning and memory in rodents and, as such, represent potential drug candidates for the symptomatic treatment of AD. SL65.0155 [5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one monohydrochloride] is a 5-HT4 receptor partial agonist with high potency and selectivity for the CNS splice variants of the 5-HT4 receptors. SL65.0155 was demonstrated to have promnesic activity in several models of learning and memory in rodents (Moser et al. 2002, JPET 302, 731-41).
- Senile plaques observed in the post mortem brain of AD patients are constituted of β-amyloid peptide. The β25-35 amyloid peptide fragment contains the 11 amino-acid (GSNKGAIIGLM) that are necessary and sufficient to induce neuronal toxicity. It exhibits large β-sheet aggregated structures and retains the toxicity of the full-length peptide. Intracerebroventricular (i.c.v.) administration of the β25-35 amyloid peptide in mice produces neurodegeneration, learning and memory deficits, and has therefore been proposed as a valid model of β-amyloid-induced toxicity.
- The aim of these studies was to assess the effects of SL65.0155 on the cognitive deficits induced by i.c.v. infusion of Aβ25-35 in different memory models in mice.




## Methods

### Animals

- C57Bl/6 or CD1 male mice weighing 28±2 g at the time of testing were used. They were fed *ad libitum* and kept in a controlled environment (12h/12h dark/light cycle, 21°C, 50% humidity).

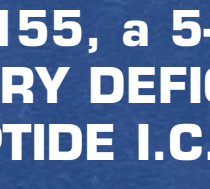
### β25-35 amyloid peptide i.c.v. administration

- Mice were anesthetized with isoflurane. The 28-gauge, 3 mm-long needle of a micro syringe was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. 3 µl of the aggregated peptide (9 nmol) were injected within 1 min. Mice were used for behavioural experiments 10 days after injection. Control mice received the same dose of the scrambled peptide (same amino acids but in a random order).

### Y-maze

- The maze is made of 3 identical arms at equal angles. When mice are allowed to freely explore the maze, spontaneous alternation behaviour consists in visiting the 3 different arms alternatively. From arm 1, the mouse goes into arm 2, and out of arm 2, it remembers that it comes from arm 1 and goes to arm 3. The mice are placed in the maze for 5 min and the alternation % is calculated as:

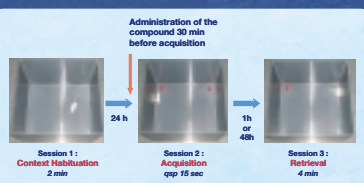
$$\frac{\text{(Total number of alternation) x 100}}{\text{((Total number of arm entries) – 2)}}$$



The Y-Maze is a test measuring spontaneous alternation as an indicator of working memory performance. Spontaneous alternation is a natural behaviour without response-reinforcement interaction.

### Object Recognition task

- The object recognition test takes place in a square open field (square : 52 cm) and consists in 3 sessions. Mice are firstly habituated to the context for 2 min, 24h prior to the acquisition. For the acquisition, mice are placed in the arena, in the presence of 2 identical objects. Animals are allowed to explore the objects until they reach 15 sec of exploration (cut-off: 5 min). After an interval (forgetting delay), mice are placed again in the enclosure containing one of the previous object and a new one. If the forgetting delay is of 1h, mice remember the familiar object and spend more time exploring the new one. With a long (48h) forgetting delay, mice don't remember the first object and spend the same time exploring both objects.




The object recognition task measures the ability of the animal to discriminate between a familiar and a novel object and is an indicator of the episodic memory performance. Object recognition is a pure memory task without response-reinforcement interaction.

## Conclusion

**SL65.0155, a 5-HT4 receptor partial agonist displayed pro-cognitive activity in two types of memory using a pathophysiological model of Alzheimer's disease in mice.**

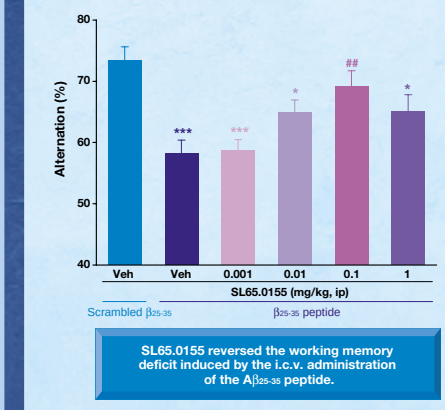
- **SL65.0155 facilitated episodic memory in the object recognition task in normal mice.**
- **SL65.0155 reversed the working memory and episodic memory deficits induced by Aβ25-35 peptide i.c.v. administration in mice.**
- **These effects were still apparent following repeated treatment, indicating the absence of tachyphylaxia.**
- **These effects were fully blocked by GR113808, a 5-HT4 antagonist, confirming the involvement of the 5-HT4 receptor in the pro-cognitive activity of SL65.0155.**
- **Taken together, these results strengthen further the involvement of the 5-HT4 receptor in learning and memory processes and confirm the potential of SL65.0155 as a symptomatic treatment of the cognitive deficits linked to Alzheimer's disease.**

**DISCLOSURE**
Authors declare that they have no conflicts of interest regarding the present results.

## Results

### SPONTANEOUS ALTERNATION

**Figure 1 EFFECT OF ACUTE TREATMENT OF SL65.0155 ON SPONTANEOUS ALTERNATION DEFICIT INDUCED BY Aβ25-35 INFUSION IN THE Y-MAZE IN MICE.**

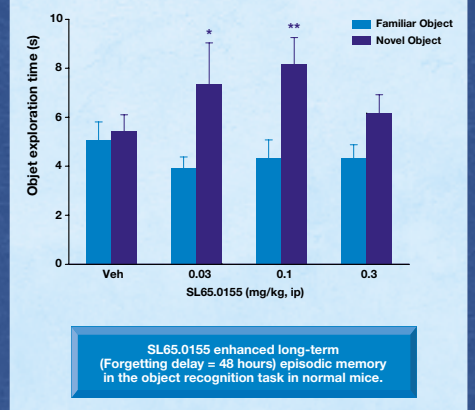



SL65.0155 reversed the working memory deficit induced by the i.c.v. administration of the Aβ25-35 peptide.

i.c.v. administration of the Aβ25-35 peptide was performed 10 days before testing and administration of SL65.0155 was done i.p. 30 min before the test.
*** p<0.001 and * p<0.05 vs. scrambled/veh and ## p<0.01 vs. β25-35/veh. n=12 mice per group.

### OBJECT RECOGNITION

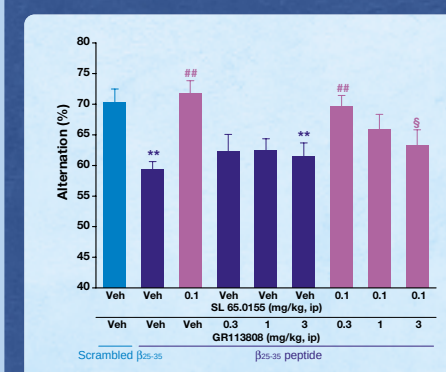
**Figure 4 EFFECT OF SL65.0155 ON LONG-TERM EPISODIC MEMORY IN THE OBJECT RECOGNITION TASK IN MICE.**




SL65.0155 enhanced long-term (Forgetting delay = 48 hours) episodic memory in the object recognition task in normal mice.

** p<0.01 and * p<0.05 vs. familiar object. The compound was injected 3 times : 30 min before each session. n=9-11 mice per group.

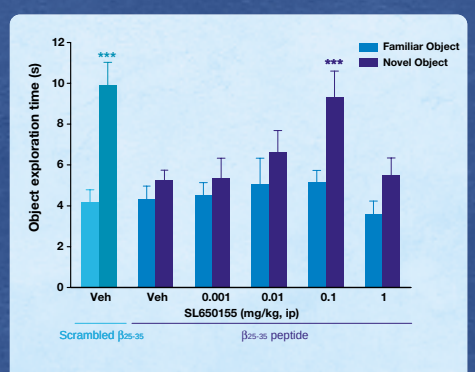
**Figure 2 EFFECT OF GR113808, A SELECTIVE 5-HT4 RECEPTOR ANTAGONIST ON THE RESTORING EFFECT OF SL65.0155 ON THE Aβ25-35-INDUCED DEFICIT OF SPONTANEOUS ALTERNATION IN THE Y-MAZE.**




The beneficial effect of SL65.0155 on the working memory deficit induced by the i.c.v. administration of the Aβ25-35 peptide is mediated by the 5-HT4 receptor since it was blocked by the administration of GR113808.

i.c.v. administration of the Aβ25-35 peptide was performed 10 days before testing. Administrations of SL65.0155 and GR113808 were done i.p. 30 min before the test.
** p<0.01 vs. scrambled/veh/veh ; ## p<0.01 vs. β25-35/veh/veh and § p<0.05 vs. β25-35/veh/0.1. n=12-16 mice per group.

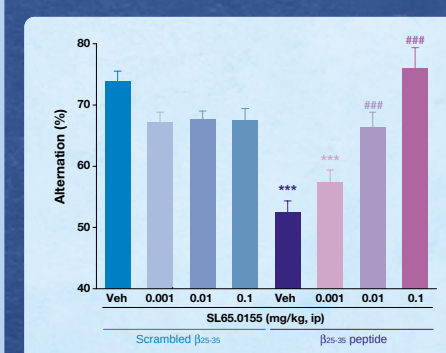
**Figure 5 EFFECT OF ACUTE SL65.0155 ON THE DEFICIT OF EPISODIC MEMORY INDUCED BY Aβ25-35 IN THE OBJECT RECOGNITION TASK IN MICE.**




The deficit of episodic memory induced by the i.c.v. administration of the Aβ25-35 peptide was reversed by an acute administration of SL65.0155.

i.c.v. administration of the Aβ25-35 peptide was performed 10 days before testing and administration of SL65.0155 was done i.p. 30 min before the acquisition (session 2).
*** p<0.001 vs. familiar object. n=11-15 mice per group.

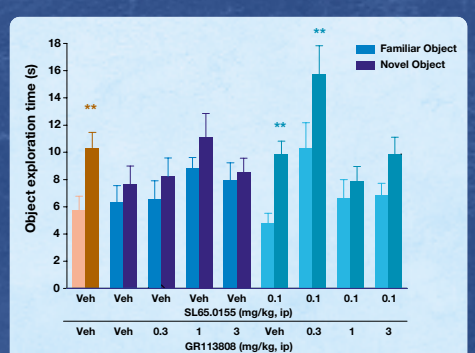
**Figure 3 EFFECT OF REPEATED TREATMENT WITH SL65.0155 ON SPONTANEOUS ALTERNATION DEFICIT INDUCED BY Aβ25-35 INFUSION IN THE Y-MAZE IN MICE.**




After repeated administration (7 administrations), SL65.0155 still reversed the working memory deficit induced by the i.c.v. administration of the Aβ25-35 peptide.

i.c.v. administration of the Aβ25-35 peptide was performed 10 days before testing. SL65.0155 was administered 7 times : 2/day during 3 days and the last one, 30 min before acquisition.
*** p<0.001 vs. scrambled/veh ; ### p<0.001 vs. β25-35/veh. n=12 mice per group.

**Figure 6 EFFECT OF GR113808, A SELECTIVE 5-HT4 RECEPTOR ANTAGONIST ON THE RESTORING EFFECT OF SL65.0155 ON THE Aβ25-35-INDUCED DEFICIT OF EPISODIC MEMORY.**




The beneficial effect of SL65.0155 on the deficit of episodic memory induced by the i.c.v. administration of the Aβ25-35 peptide is mediated by the 5-HT4 receptor since it was blocked by the administration of GR113808.

i.c.v. administration of the Aβ25-35 peptide was performed 10 days before testing. Administrations of the compounds were done i.p. 30 min before the acquisition (session 2).
** p<0.01 vs. familiar object. n=11-15 mice per group.